January 12, 1953

Dear Bruce:

I shall send the strains requested as soon as I can get around to it. I believe I did not send SW-926, but substituted SW-938 instead. SW-926 is abony —x SW546; SW-938 is SW-546 —x abony. As the mother strain in the latter was already diphasic, it seemed to me a better exemplification of the anomalous $H_1^{1.2}$ and more likely to have a straightforward subsequent behavior. The origin of the 1,2 phase of SW-546 is still somewhat of a mystery. I have not been able to repeat the isolation of an enx:1,2 from abony —x 546, perhaps because the 546 would have to be in a sort of cryptic second phase for the substitution to be effective.

Are we still at cross-purposes about SW-674? (Or do you have some other reference for S. dublin(?) O in your letter?) SW-674 is typhimurium to which gp has been transduced in place of i; I shall be very surprised if it phage-types as dublin. You should find the other characters of SW-435 (nutrition, Xyl- Gal- S^r) still intact also.

I didn't intend to stir up such a hassle about the authorship of the paper-- and anticipated no personal difficulties at all. I won't restate my own views, and will accede entirely to your own decisions on the matter. It was to avoid the tiresome necessities of divided leadership and responsibility that I raised the point in the first place, but I suppose it will make very little difference in the end. I hope you will go ahead as if this is your own paper, though of course you will tend to the superb advice you get later from your colleagues. At any rate, you ought not to delay the actual writing on any account, as it is impossible to suggest revisions until one can see the whole. I agree with you that this paper must be addressed to the microbiologists, and the genetics confined to the least necessary (which in this case is also the most) to make it intelligible. I think one could go so far as to say that the suggestion of linkage has been partly confirmed (or at least the stated alternative disqualified) by subsequent backcross tests. I doubt if the facts yet justify a more sophisticated discussion at any level! Norton's objection on suppressors is at least partly semantic, as to what one means by suppressors vs. Fla loci. It is true that the different Fla- mutants have not been tested in a proven uniform background, and that they might show different interactions in other combinations. This would in no case disqualify them as worthy of being called "Fla-", and the reservation of interactions with the residual genotype should be implicit in any discussions of gene action. In any given test, whatever locus is altered to restore motility was, by this definition, a Fla-.

I should rather not, just yet, send out any heterozygous diploids. Except tossomeone who is prepared to wallow in the whole morass, their individual behavior is so complex as to lead to serious misinterpretations. I am not concerned about your falling into such a trap, but fear that you would be embarrassed by requests from other quarters. However, I have been meaning anyhow to send you SW 684, Gal+/- from PLT22—x666. This is, I think, also undoubtddly a segregation, and rather resembles the behavior of the E. coli diploids (except, of course, that just one character is involved). I don't quite see what you're doing with the iodo-eosin analogues. I'll be happy to send you some of our eosin, but suggest that you simply write around for samples till you find a

batch that suits you. If my eosin doesn't work with your Methylene Blue, that'll be your next step. You may have to vary the eosin: MB proportions slightly from the indicated ratio of 6:1, by empirical tests. This sounds like a lot of trouble, but the result (for years to come) is still worth it.

I hope we can avoid a delicate situation on the publication of the serology. I have been cultivating Edwards' good offices for some time, and am deeply indebted to him for innumerable favors. We have had, I think, an understanding that he would collaborate on the detailed serological work. I suggest that you get Joan Taylor's critical data as a sort of addendum (perhaps under her authorship) to the present paper. In the text it might also be indicated that Edwards has confirmed the initial diagnoses. I will have to wait to see Edwards about further developments, but expect we will collaborate closely on further generation of serotypes: I am hoping, in fact, that this part of the work itself will be continued largely at Chamblee, except for my own studies which will be more intensive than extensive, at least anent phase-variation. I do not think that Edwards' propietary interest will be infringed, however, by a publication in the form just suggested. Can you think of any easier solution? I would hesitate to suggest to two other people that they collaborate with each other until I have a much better personal insight.

Thanks for the German references: I'll look them up and send you English abstracts. While we're at it, I noticed Andrewes' comment (1925) that variation was more frequent in broth than on agar. If he had any real observations on this, it is hard to see how they could be fallacious. By the by, in his 1922 paper he refers to his own infection with typhimurium, which was substantially pure group phase (anent p. 411 your paper). In considering such questions, the purity of the antibody response might be even more critical than necessarily limited isolations. But anyhow, do you think Andrewes' comment on broth vs. agar could be right? I have always been a little skeptical of your explanation of binns; perhaps these are strains which are unusually stable on agar. At any rate, this seems like the most tangible lead on the control of phase variation. On the activation-shift hypothesis, experimental control should be possible.

I can't be sure whether you'd gotten mine of the 25th before you sent yours of the 29th. To avoid such uncertainties, it might be a good idea to include the formality of reference to previous letters rec'd (or is this too stuffy?) For reasons similar to yours, there has been a temporary decrescendo, and I don't know whether we shall pick up much before packing off for Chamblee (ETD: Jan. 25). Some minor nuggets: The host-adaptation of PLT22 is confused. The relative e.o.p. (typhimurium;LT2: paratyphi B SW666) goes as follows: PLT22 itself ca. 7, adapted to SW666 ca. -2, readapted to LT2, 4. About the same holds for the lytic variant,22V. Several, but not many separate lines were tested for the above, with consistent results. As the adaptation is not completely reversible, there may be both an induced phenotypic effect and a selection of spontaneous mutants. SW666 itself carries another phage, and it is an amusing possibility that the adaptation is partly a phenotypic blending with this phage. Efficiency of transduction is qualitatively similar to the r.eop., but not quantitatively. E.G., PLT22 has ca. 1% FA on 666 as on LT2; most of the transductions here are not lysogenic, Ad.22V does not appear to induce lysogenicity in SW666 (unfortunately, as some interesting substitution expts. would have been possible with such a marker), although its plaques here are quite muddy. I have had some i's from LT-2^{1,2} —x SW666; have not yet followed them through A fairly clean preliminary expt. using 22V to identify infected bacteria was completed (see last letter). It agrees quite well with the proposition that all (or at least almost all) transductions occur to bacteria infected with temperate phage. As the number of transductions is limited by the amount of phage, it seems unlikely that phage-infection is simply an auxiliary condition. (Some more expts. on this

point, viz. the yield of transductions at multiplicities below saturation with various mixtures of Gal+ and Gal- FA, may be needed. They may add up to rather substantial proof that FA is carried by the same particles as carry lysogenizing activity, i.e., that FA = phage not only qua skins, but also qua contents.

I've done just a few experiments with SL-13, and can confirm getting a and i —x PLT22. FA from paratyphi B has so far had no effect, and the yields in all cases have been very small. I thought this might be due to roughness, but the overall somatic antigen seems well developed. I should do some adsorption expts., and will. Para A was an important type to decide on the role of XII_2. Do you have any explicit information on the presence of this component; in SL-13? So far, its susceptibility to transduction has been so low as to discourage any extensive work. We may really have to buckle down to isolating a good many new mutants (Fla-) from some standard strain. I've thought of LT-1 (as the only LT I found susceptible to Chi phage). I'll send you anything promising that may come of this.

Spicer's still up to attempting somatic antigen transductions. There are many enough technical difficulties; it still hasn't worked. Have you done any more with techniques for classifying O colonies? I should think one could add ~~xxxxxxxx~~ ~~xxxxxxxxx~~ something like methylcellulose to increase the viscosity of motility agar, and make it an indicator rather than a selective medium. So far, have had very sloppy results trying to grow colonies at low temperature and let them migrate briefly at higher.

I sent some reprints off to you; you will recognize the semicolons in some as your own. Your critical judgment was invaluable, and I did not want to leave this unsaid. Page proof has only just come in for the L&L lysogenicity opus of which you have a preprint. In case anyy references are needed, it turns out Genetics 38:51-64 ("Jan." 1953).

Sincerely,

Joshua Lederberg

P.S. Sagebiel grew S. typhi in chloromycetin broth. In concentrations over 1 ug/ml the cells were immotile and H-inagglutinable. The Vi response also dropped with more than about 2 mg/ml, while O-agglutinability increased. He did not reinoculate into plain broth! so your question's not answered. It would be easy enough to do. He made up his stock chloromycetin in propylene glycol. I don't know why.
The other paper you quoted is in the bindery just now.

I hope I've given you the proper genealogy of SW-534. SW-703 is Edwards' #3, and cannot be the ancestor, if only on grounds of inositol and rhamnose fermentation, as well as diphasicity. Edwards #157 was represented as SW-546, and certainly is the ancestor of SW-534 etc. I will send you SW-703, but the allegation that it is the parent is incorrect. Perhaps this is what you meant, and hope to have Felix exclude 703, as I am sure he will.

JL